EXPERIMENTAL SPOROTRICHOSIS, AMBIENT TEMPERATURE AND AMPHOTERICIN B*

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Intravenous injections of *Sporotrichum schenckii* to mice produced miliary lesions in several internal organs, especially in the liver, and in the muscles of the hind legs when the animals were kept at a low ambient temperature of 2-5°C. Mice kept at 13-17°C. developed lesions in the muscles of the hind legs but not in the liver and internal organs.

Amphotericin-B, 0·04 mg., daily, intraperitoneally for 10 days prevented the myositis in the animals kept at 13-17°C. but not in those kept at 2-5°C.

It has been shown that low ambient temperatures favour the development of some experimental deep mycoses (Mackinnon, Conti-Díaz, Yarzabal & Tavella, 1960; Conti-Díaz & Mackinnon, 1961; Mackinnon & Conti-Díaz, 1962a, 1962b; Mackinnon, 1963). We presumed that this effect may influence the dosage of antifungal antibiotics and chemotherapeutic compounds necessary for cure or amelioration in man and animals.

The following experiments constitute an attempt to demonstrate the existence of combined effects of amphotericin-B and ambient temperature on an experimental mycosis.

METHOD AND RESULTS

Strain IHM 1463 of *S. schenckii,* isolated from a case of sporotrichosis in Uruguay was used. A total of 52 male "Swiss" white mice weighing 18 to 22 g. were injected intravenously with 0·3 ml. of a suspension of yeast cells in isotonic saline from 4 day old yeast phase cultures grown on blood agar at 37°C. The suspension had a density of grade 2 on the MacFarland scale. The inoculated mice were distributed in 6 groups. Groups 1, 2 and 3 were placed in a room at 2-5°C and groups 4, 5 and 6 were kept at 13-17°C. Amphotericin-B was administered intraperitoneally in daily doses, 0·2 mg. to groups 1 and 4 and 0·04 mg. to groups 2 and 5, dissolved in 0·2 ml. of isotonic saline solution. The mice of groups 3 and 6 (controls) received 0·2 ml. of the saline solution without the antibiotic. The treatment was continued for 10 days.

After 15 days all the survivors were killed, 2 pieces of liver and spleen were removed from each, placed on Sabouraud glucose agar slants and incubated at 28°C for 16 days. The weight of the 2 pieces of liver was approximately 0·3 to 0·5 g.

During the course of the experiment 10 mice died. They had all received the larger dose of amphotericin-B and showed an irritative peritonitis caused by the antibiotic. Mortality was higher at 2-5°C than at 13-17°C. No mice died in the other groups. The results are summarized in table 1. In the animals kept at 2-5°C and not treated with the antibiotic the liver showed very numerous small miliary lesions like those described by Tsubura & Schwarz (1960). Similar lesions were also seen in some animals in the spleen and heart and around and close to the testicles. In the

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animals kept at 13-17°C no lesions were visible in the liver, spleen and heart but they were visible around the gonads. Miliary lesions were seen in the muscles of the buttocks and hind legs in the animals kept at 2-5°C and at 13-17°C. The table shows that the dose of 0·04 mg. of amphotericin-B prevented the myositis in the animals kept at 13-17°C but not in the animals kept at 2-5°C.

Complementary experiment

It might be presumed that the antibiotic accumulated in the peritoneum and on the surface of the liver of the treated mice would prevent the development of the fungus on the agar slants. The following experiment allows this possibility to be discounted.

A total of 12 uninoculated mice were given the larger dose, 0·2 mg., of amphotericin-B intraperitoneally in 0·2 ml. isotonic saline solution for 10 days. Six mice were kept at 2-5°C and 6 at 13-17°C. One of the former and 3 of the latter died. The 8 survivors were killed 15 days after the initiation of the experiment; pieces of liver were removed and placed on Sabouraud glucose agar slants. A small amount of a yeast phase culture of *S. schenckii*, strain IHM 1463, was placed over the pieces of liver and the fungus grew readily in all the tubes.

Comment

The results show an effect of ambient temperature on the disease of the mice inoculated intravenously with *S. schenckii* which is most interesting because we kept the animals at the moderate ambient temperatures common in buildings without heating and air conditioning devices. In mice kept at 2-5°C miliary lesions were seen in several organs, specially in the liver, and in the muscles of the hind legs. At 13-17°C lesions of the liver were not visible to the naked eye but lesions in the muscles of the legs were visible. In a previous unpublished experiment we kept 5 inoculated mice at 2-5°C and 6 at 28°C; the former died in 5 to 14 days while the latter survived and after 21 days they showed neither the myositis nor the hepatitis or any other visceral involvement excepting a few miliary small lesions around the gonads. We may infer that the results recorded will differ in the same laboratory at different seasons of the year and in between laboratories in regions with different climates.

Another interesting feature is that a dose of amphotericin-B which prevented the myositis in animals kept at 13-17°C did not show the same efficiency in the animals kept at 2-5°C. At the lower ambient temperature a larger dose of the antibiotic was necessary to obtain a similar effect. Unfortunately the infection was not eradicated, positive cultures were obtained at autopsy from many treated mice (table 1) and it is impossible to foresee what the evolution

Mg. of amphotericin-B Daily dose	*With lesions in the liver*		*Cultures from the liver*		*Myositis*	
	2-5°C	13-17°C	2-5°C	13-17°C	2-5°C	13-17°C
0·00 mg.	8/8*	0/7	8/8	7/7	8/8	6/7
0·04 mg.	5/9	0/10	9/9	9/10	9/9	0/10
0·2 mg.	0/2	0/6	0/2	3/6	0/2	0/6

* Numerator=Number of positive; denominator=number of survivors at the end of the experiment.

Table 1.—Results of Treatment of Mice Infected with *Sporotrichum schenckii*

of the remaining infection would have been had the mice not been killed on the 16th day. Short experiments are preferred in order to minimize the influence of the immunological response of the host, especially as a synergistic action between immune globulin and amphotericin-B was demonstrated by Gordon & Lapa (1961) in cryptococcosis and by Gordon & Gruft (1962) in North American blastomycosis. The enhancement of the antifungal effects of amphotericin-B might be possible by different methods.

Increasing the effectiveness of amphotericin-B or other drugs by adjusting ambient temperature has not very important practical implications in the treatment of sporotrichosis because this disease is easily treated with iodides and because according to Mackinnon & Conti-Díaz (1962a) and to Galiana & Conti-Díaz (1963) it can be treated by the use of local heat alone. On the other hand, already published experiments show that high ambient temperatures inhibit or retard the development of several other deep severe mycoses: cryptococcosis (Kuhn, 1949; Kligman, Crane & Norris, 1951), South American blastomycosis (Mackinnon, Conti-Díaz, Yarzabal & Tavella 1960), North American blastomycosis (Conti-Díaz & Mackinnon, 1961) and histoplasmosis (Mackinnon & Conti-Díaz, 1962a). The inhibitory effects of amphotericin-B and some other drugs on some fungi might be influenced by the thermal conditions of the involved tissues and organs.

Resumen

La inoculación intravenosa de *Sporotrichum schenckii* a lauchas produjo lesiones miliares en varios órganos internos, especialmente en el hígado, así como en los músculos de las patas posteriores cuando los animales fueron colocados en ambiente a baja temperatura: 2-5°C. En las lauchas en ambiente a 13-17°C. se desarrollaron lesiones en los músculos de las patas posteriores pero no en el hígado y órganos internos.

La anfotericina-B en dosis diaria intraperitoneal de 0·04 mg. administrada durante 10 días impidió la miositis en los animales colocados a 13-17°C. pero no en los colocados a 2-5°C.

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